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White Grub in Bluegill in the Sangamon River: Impact of Sewage Effluent and Flow Regime on Infection Parameters

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White grub in bluegill in the Sangamon River:

Impact of sewage effluent and flow regime on infection parameters

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Miranda White

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Abstract

White grub (*Posthodiplostomum minimum*) is a digenetic trematode that uses a piscivorous bird, a pulmonate snail, and a centrarchid fish to complete its life cycle. To assess the impact of impoundment and sewage effluent on white grub infection parameters, infections in bluegill (*Lepomis macrochirus*) were monitored in 3 reaches of the Sangamon River near Decatur, Illinois: Reach 1- free-flowing river upstream of Lake Decatur Dam, unimpacted by sewage effluent, Reach 2- impounded flow below Lake Decatur Dam, unimpacted by sewage effluent, and Reach 3- free-flowing river downstream of Sanitary District of Decatur, impacted by sewage effluent. Liver and kidney abundances of white grub were compared to bluegill condition indices to assess effects of parasite burdens on the intermediate fish host. Prevalence of white grub in the Sangamon River was 96.4%, with a mean abundance of 153.7 metacercaria per individual. White grub abundance was highest in Reach 2, followed by Reach 1, and lowest in Reach 3. In low level infections (<50 metacercariae), white grub had a significantly higher ratio of liver to kidney abundance, whereas bluegill with medium/high (>50 metacercariae) levels of infection, liver and kidney abundances were almost equal. Most condition indices (Relative weight, gonadosomatic index, and cortisol baseline levels) did not correlate with parasite burden. However, Fulton's condition score in small bluegill, typically with low level infections, was positively correlated with parasite burden.

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Introduction

Parasites can have negative effects on the host by altering behaviors, reducing growth and/or fecundity, and affecting morphology (Marcogliese, 2004). However, the effects of parasites on their hosts can be compounded when the habitat is degraded, leaving the host in poorer condition when compared to a non-parasitized host (Marcogliese, 2005). In polluted habitats, it is predicted that the host's immune system capabilities are reduced, rendering them more susceptible to parasites (McDowell et al., 1999). In turn, parasites themselves, especially those with indirect lifecycles, may be affected by polluted habitats, because the degraded environment may decrease host populations (Lafferty, 1997) or directly affect parasite stages (Hudson et al., 2006; Lafferty, 1997; Marcogliese, 2004).

Sewage effluents are common contributors to aquatic habitat degradation, since they lead to an increase in suspended solids, organic matter, phosphorus, and nitrogen (Kreeger, 2000). Effluent streams can cause drastic changes in fish (Paller et al., 1983) and invertebrate communities (Bendell–Young et al., 2000), as well as trematode prevalence (Huspeni et al., 2005). Aquatic macroinvertebrates, such as mollusks and arthropods that are intermediate hosts for parasites, can be sensitive to habitat degradation and pollution, and if these populations decline, it is likely that the parasites they vector will decline as well (Landsberg et al., 1998).Therefore, parasites of fish, especially parasite species with complex life histories, may provide useful information regarding habitat degradation and quality (Landsberg et al., 1998).

For example, digenetic trematodes are common parasitic platyhelminth worms (Janovy et al., 1997) that alternate sexual reproduction in a definitive vertebrate host with asexual reproduction in an intermediate snail host (Figure 1) and many species include an additional life stage as encysted metacercaria in a third host species (Dogiel et al, 1961). Thus, since the presence of this parasite requires all 3 hosts, impacts to any of them should affect parasite populations. Conversely, high numbers of the parasite should reflect healthy host populations.

Posthodiplostomum minimum (MacCallum, 1921; Dubois, 1936), commonly known as white grub, is a digenetic trematode common in aquatic systems in the Midwest. It uses a great blue heron (*Ardea Herodias*; Linnaeus, 1758) as a definitive host (Dogiel et al., 1962; Hoffman, 1967), a pulmonate snail usually from the genus *Physa* (Fitizinger, 1833) as first intermediate host, and typically bluegill sunfish (*Lepomis macrochirus*; Rafinesque, 1819) as a second intermediate host. Adult flukes, in the intestines of herons, produce eggs which are passed in the bird's feces into water (Lane and Morris, 2000). Ciliate larvae (miracidia) hatch out of the eggs and enter the snail host whereupon they shed the cilia and develop into sac-like sporocyst larvae (Miller, 1954). Specialized cells in the sporocysts then give rise to a second larval generation (rediae) (Miller, 1954) and reproductive cells inside the rediae give rise to motile larvae (cercariae) (Dogiel et al., 1961). Cercariae leave the snail and find the second intermediate host, a fish in the Centrarchidae family (Hoffman, 1967). After burrowing through the skin of the fish, cercariae shed their tail and migrate through tissues of the fish host (Hoffman, 1967; Hunter and Hunter, 1940), eventually developing into encysted metacercariae in the liver, kidney, heart, and spleen (Colley and Olson, 1963; Hoffman, 1967; Wilson et al., 1996). Unless fish are able to develop resistance to reinfection by metacercariae or eliminate infections, parasites will accumulate over time and remain viable until the fish host is consumed by the respective definitive bird host (McKeown and Irwin, 1997). Once thought to be a generalist parasite, recent taxonomic work indicates that white grub is actually a complex of cryptic species with varying levels of fish host specificity (Marcogliese, 2016). Bluegill in Illinois and Indiana are most commonly infected with white grub subspecies 3 (Marcogliese, 2016; Boone et al., 2018).

Although effects of parasites on intermediate fish hosts are often unknown, several have been shown to have direct effects through alteration of host physiology, reproduction, or growth (Sousa, 1991). Often, these effects are dose-dependent, meaning those individuals that are highly parasitized, especially if suffering from malnutrition, are more likely to experience extreme negative consequences as opposed to uninfected individuals or those with low parasite burdens (Roberts and Janovy, 2000). In the case of white grub, as cercaria penetrates and migrates through fish host tissues (Pracheil and Muzzal, 2009) it causes minor damage and external hemorrhaging (Dogiel et al., 1961; Meade and Bedinger, 1967). After transformation into metacercariae, the risk of further damage decreases significantly unless a substantial number of metacercariae accumulate and the mass of parasite material interferes with the host's metabolism, resulting in mortality (Dogiel et al., 1961; Hoffman, 1967; Meade and Bedinger, 1967).

Bluegill have been repeatedly shown to live with high infection levels of white grub (Colley and Olson, 1963; Steinauer and Font, 2003; Welsh et al., 2006). However, it is unclear whether a high rate of white grub parasitism can affect the condition and survival of the fish host (Sillman, 1957; Hoffman, 1967), especially in impacted aquatic systems. Indices such as Fulton's condition, relative weight (W_r), gonadosomatic index

(GSI), and cortisol levels have been used to asses fish condition and stress level (Wright, 2000; Copeland et al., 2008; Noble and Noble, 1961).

Fulton's condition is "the ratio between a fish's weight and length raised to the third power multiplied by 10^5" (Blackwell et al., 2000). Fulton's condition assumes fish growth is isometric, therefore, fish in good condition are heavier at any given length (Ricker 1975). Relative weight (W_r) is a common measure of fish condition, defined as the "ratio of actual weight of a fish to what a rapidly growing healthy fish of the same length should weigh" (Wright 2000). Relative weight can be classified into three different categories: 1) a value less than 0.8 is considered poor or severely thin, 2) values between 0.8 and 1 are within the range for a healthy population while not ideal, 3) values greater than 1 are considered to not be using the resources sufficiently (Wright, 2000). For bluegill, Fulton's condition is used for individuals 79mm or shorter, whereas relative weight is calculated for individuals 80mm or longer (Wright, 2000).

Fulton's condition and relative weight assess the weight of an individual at a given length, whereas the gonadosomatic index (GSI=ratio of fish gonad weight to body weight) assesses fecundity. Although the cause of initiation of gonad development in juvenile male bluegill is unknown, it is likely due to the presence of females, which in turn may cause gonad development in females due to the pheromones secreted by mature males (Aday et al., 2003). The gonadosomatic index and relative weight (W_r) both positively correlate with the breeding season (Copeland et al., 2008).

Cortisol baseline levels, based on production of adrenocortical hormone (ACTH) from the pituitary gland and consequent release of adrenal glucocorticoids, are commonly used as indicators of stress response in fish (Noble and Noble, 1961). Female fish will typically exhibit a higher level of stress-induced cortisol than males, however, cortisol concentrations show considerable intra-individual variation (Cook, 2012). Cortisol concentrations can be used to show if parasite burdens are causing a stress induced steroid hormonal effect on fish intermediate hosts (Mommsen et al., 1999).

While monitoring effects of the Sanitary District of Decatur effluent stream on the Sangamon River, preliminary studies of fish showed heavy parasite burdens of white grub in bluegill. The Sangamon River is impounded in several locations and the Sanitary District of Decatur discharges treated effluent approximately 4.5 km downstream of Lake Decatur Dam. Changes in flow regime associated with the dam and stressors associated with point source pollution from the Sanitary District of Decatur provide a unique opportunity to further investigate the interactions of environmental stressors and parasites. This project uses white grub in bluegill in the Sangamon River to assess impacts of impoundment and sewage effluent on infection parameters, and evaluate how parasite burdens affect parasitized fish in an impacted system.

Materials and Methods

Fish sampling

A total of 427 bluegill was collected in spring 2013 (May), fall 2013 (October, November), and spring 2014 (April, May, June) from three reaches of the Sangamon River in Decatur Illinois: Reach 1- free-flowing river upstream of Lake Decatur Dam, unimpacted by sewage effluent, Reach 2- impounded flow below Lake Decatur Dam, unimpacted by sewage effluent, and Reach 3- free-flowing river downstream of Sanitary District of Decatur, impacted by sewage effluent (Figure 2). Bluegill were also collected from Reach 2 in fall of 2012 (September, October). Fish were captured by multiple means (AC electrofishing, DC backpack shocker, hook and line, hoop nets, and minnow fyke nets) to ensure an adequate sample size with access to a wide standard length range. Specimens were individually frozen in Ziploc® bags prior to dissection.

Fish dissection/Parasite abundance analysis

Bluegill were thawed and the total length (mm) and weight (g) were recorded prior to dissection. The kidney, liver, and gonads were removed and the wet weight of each individual organ was obtained. The sex of all mature individuals (age one fish and greater) collected in the spring seasons were recorded. In spring 2014, some bluegill did not have measurable gonads, by late June most had already reproduced, and were assigned a gonad weight of zero (6 bluegill).

Liver and kidney tissues were mechanically sheared separately in a 0.9% saline solution to facilitate maximum extraction of white grub metacercariae. In organs exhibiting low levels of infection, all parasite metacercariae were counted to determine abundance (number of parasites in both infected and uninfected hosts). In organs exhibiting high levels of infection, metacercariae were suspended in 10ml to 15ml volumes and the average of three 1ml aliquots, corrected back to original suspension volume, was used to approximate abundance. Abundance of white grub was not normally distributed (skewness=5.8685, kurtosis=47.1431, D=0.3273); therefore, parasite abundance was normalized by log transformation (skewness=-0.0702, kurtosis=-0.6365, D=0.0553). Since sample size of bluegill was large, log transformation was acceptable for normalization. After parasite necropsy, otoliths were removed, placed in mineral oil, and two independent readers estimated age using a dissecting scope.

Bluegill cortisol levels

In spring 2014, blood cortisol baseline levels were calculated using blood removed from gills with 75mm heparinized capillary tubes within three minutes, while blueguill were still in electro-anesthesia. Blood samples were kept on ice for no more than six hours. Capillary tubes were spun at 10,000 rpm for 3 minutes in a micro-hematocrit centrifuge. The serum from each individual was removed using a micro-hematocrit capillary pipette bulb, placed in separate 0.6 mL microcentrifuge tubes, and kept at -80^{o}C. Fish were processed through parasite necropsy before cortisol levels were determined using an ELISA Kit from Enzo Life Sciences. Although 29 bluegill (Reach 1-14, Reach 3-15) had blood samples, only 16 had enough serum to calculate cortisol baseline levels, 4 of those blood samples were combined with another serum sample from bluegill in the same reach with like white grub abundance. In this case, no more than two serum samples were combined to obtain one cortisol baseline level.

Statistical Analyses/ Condition Indices

Parasite prevalence and mean abundance were calculated for each reach, season, bluegill age class, and organ (liver and kidney). Prevalence is defined as the percent of individuals infected, whereas mean abundance is the average number of parasites per individual (infected and uninfected) (Bush et al., 1997). Pearson's Correlation coefficient was used to compare bluegill length (mm) to white grub log abundance.

Average length (mm) at age of bluegill was analyzed using an ANOVA. Fish condition was determined using relative weight (W_r = (actual fish weight/standard weight for fish of the same length) x 100) described by Murphy et al. (1991) and Blackwell et al. (2000) for bluegill 80mm or larger or Fulton's condition (K = (weight in grams/length in millimeters cubed) x 100,000) used by Neff and Cargnelli (2003) for bluegill 79mm and smaller. Gonadosomatic Index (GSI) was calculated as the percent of gonad weight to total body weight. Condition factors (Fulton's condition, relative weight, gonadosomatic index, cortisol baseline levels) were compared to white grub log abundance using Pearson's Correlation coefficient.

Differences in prevalence among reaches, age classes, and seasons were compared using logistic regression and chi-square analyses. White grub log abundance was compared to the Sangamon River water discharge (3/sec) recorded from USGS gauging stations at Monticello (Reach 1), Route 48 (Reach 2), and Riverton (Reach 3) using Pearson's Correlation Coefficients. Significance was set at p=0.05 and no corrections were made for multiple comparisons. All Pearson's Correlation coefficients and ANOVA analyses were performed with the statistical package Statistical Analysis

System (SAS), whereas logistic regressions and chi-square analyses were conducted using the program Statistix.

Since bluegill were removed only from Reach 2 in the fall of 2012, this sampling data was not included in analyses that compared all three reaches together. However, these data were included in the comparisons that analyzed bluegill parasitism rates in Reach 2 only, such as infection levels in bluegill of different age classes and seasons.

Results

Fish Demographics

A total of 427 bluegill was collected, with an age class range of 0-5, from three reaches over four seasons (Appendix 1). There was a significant effect of reach on fish size ($F(2,424)=37.97$, $p<0.0001$) and age ($F(2,424)=37.97$, $p<0.0001$). On average, individuals collected from Reach 2 were older and larger than bluegill from Reach 1 or Reach 3.

Bluegill in Reach 2 were significantly larger on average at age classes zero ($F(2,23)=45.09$, $p<0.0001$), and one ($F(2,158)=17.81$, $p<0.0001$). In age class two, bluegill in Reach 2 and Reach 3 were significantly larger on average than Reach 1 ($F(2,116)=9.10$, $p=0.0002$). Bluegill in age class three at Reach 1 and Reach 3 were significantly larger on average than Reach 2 ($F(2,95)=11.01$, $p<0.0001$), At age class four, bluegill in Reach 2 and Reach 3 were larger than bluegill in Reach 1 ($F(2,16)=5.38$, $p=0.0147$). Bluegill in age class five were only found in Reach 2 (Figure 3).

Parasite Parameters

Prevalence

Prevalence of white grub in all bluegill collected from the Sangamon River, Decatur, IL was 96.5%, and was significantly affected by reach (Co=-0.54, CoSe=-3.51, $p=0.0004$) and fish age (Co=0.99, CoSe=-2.56, $p=0.0104$). Although white grub prevalence did not show a seasonal difference overall (Co=0.53, CoSe=1.40, $p=0.1608$), Reach 3 prevalence in spring 2013 was significantly less than expected (df=2, $X^2=10.86$, $p=0.0044$) (Figure 4).

Prevalence of white grub in combined age classes from Reach 1, Reach 2, and Reach 3 was 99%, 98%, and 91% respectively, and was significantly higher at Reach 1 and Reach 2 than Reach 3 (df=2, X^2= 14.55, p=0.0007) (Figure 4). Reach 1 had a significantly higher prevalence than Reach 3 at bluegill age class zero (df=1, X^2=7.78, p=0.0053). There were only 4 bluegill in age class zero collected from Reach 2; therefore, this small sample was removed from analysis. Bluegill in age class one was significantly higher at Reach 1 and Reach 2 than Reach 3 (df=2, X^2=13.40, p=0.0012). Prevalence at bluegill age classes two (df=2, X^2= 1.30, p=0.5222) and three (df=2, X^2=0.70, p=0.7058) did not differ significantly among the reaches. All bluegill in age class four were infected. Figure 4

Abundance

Since white grub prevalence was close to 100%, abundance and intensity were nearly identical. Therefore, abundance was used to include the few uninfected bluegill collected. White grub log mean abundance was positively correlated with length (mm) (r(425)= 0.74, p<0.0001) and significantly increased with each bluegill age class (F(4,418)=128.98, p<0.0001) (Figure 5) Bluegill in age class 5 were found only in Reach 2 (N=4); due to low sample size, bluegill in age class 5 were not included in this analysis. Reach 2 had a significantly higher white grub log mean abundance than Reach 1 and Reach 3 (F(2,405)=26.05, p<0.0001).

Within each age class, white grub log mean abundance did not differ by reach at age classes zero (F(2,23)=0.87, p=0.4296), two (F(2,116)=1.51, p=0.2248), three (F(2,95)=0.37, p=0.6927), or four (F(2,17)=1.71, p=0.2088) (Figure 6). For bluegill in age class one, white grub log mean abundance was significantly higher at Reach 2, followed by Reach 1, and lowest at Reach 3 (Figure 6).

Seasonal log mean abundance patterns varied among reaches (Figure 6). At Reach 1, log mean abundance was significantly higher in spring 2014 and spring 2013 than fall 2013 ($F(2,149)=16.77$, $p<0.0001$). At Reach 2, log mean abundance was significantly higher in fall 2012 than in spring 2013, fall 2013 and spring 2014 ($F(3,144)=7.66$, <0.0001). At Reach 3, log mean abundance was significantly higher in fall 2013, than spring 2013 or spring 2014 ($F(2,124)=107.70$, $p<0.0001$) (Figure 6).

Overall, white grub log abundances in bluegill liver and kidney were positively correlated ($r(425)=0.91$, $p<0.0001$) (Figure 7). However, in low level infections (<50), the ratio of liver to kidney metacercaria was significantly higher than in than bluegill with medium (50-500) or high (>500) levels of infection (Figure 7, $F(2,424)=13.45$, $p<0.0001$).

Parasite abundance and Fish Condition

White grub log abundance positively correlated with Fulton's condition ($r(210)=0.32$, $p<0.0001$) (Figure 8). However, white grub log abundance did not correlate with bluegill condition as measured by, relative weight (W_r) ($r(186)=0.09$, $p=0.2379$), gonadosomatic index ($r(124)=-0.05$, $p=0.5556$), or cortisol baseline levels (pg/ml) ($r(10)=-0.26$, $p=0.4052$) (Figure 9).

Parasite and Environmental Conditions

White grub log mean abundance may be affected by water discharge. Sangamon River water discharge (c3/sec) was significantly negatively correlated with white grub log mean abundance ($r(10)= -0.64$, $p=0.0449$) (Figure 10).

Discussion

Both prevalence and abundance were affected by site and bluegill age class. Infection prevalence in combined age classes exceeded 95% overall, but there were differences among reaches and bluegill age classes. Although prevalence was lowest overall in Reach 3, a high proportion (>80%) of fish from all sites were infected before reaching age class 1. The few fish not infected were essentially all infected by the time they reached age class 2. Because metacercariae can remain in the viscera and organs for at least four years (Hoffman 1967), it is impossible to determine when an individual fish was infected, i.e. prevalence in age class 4 fish reflects exposure over their entire life.

I could not unequivocally determine whether fish were actually becoming infected in the given collection reach or migrating from other reaches. However, since bluegill are not thought to move extensively (Gunning and Shoop, 1963) and the reaches are both functionally and geographically isolated (upstream site separated from below the dam site by dam and approximately 17 km of river stretch), migration was considered unlikely. The slightly lower prevalence in young fish at Reach 3 may reflect some effect on parasite transmission, but it is difficult to assign biological significance at these high parasitism levels.

Abundance of white grub generally followed trends in prevalence, and was positively correlated with length and age. In agreement, fish size has been shown to be positively correlated with white grub intensity in bluegill in other studies (Colley and Olson, 1963; Steinauer and Font, 2003; Noble and Noble, 1961). As seen with prevalence, mean abundance at the three reaches differed until age class 2. Although age class 1 fish from Reach 3 had lower numbers than other reaches, fish continued to accumulate parasites as they aged, and as bluegill entered age class 2, log mean abundance became more similar. Lane and Morris (2000) reported that larval fish organs can compress, and result in death if the organs are damaged, when metacercaria levels are high. It is possible that heavily infected fish are dying in Reach 3 at a greater rate than other reaches. These infection patterns agree with other reports (Fellis and Esch, 2006; Pracheil and Muzzal, 2009) which suggested that metacercariae of white grub accumulate over time and are not expelled by the fish host. Although it is not obvious, there may be a maximum capacity of metacercaria that bluegill can sustain. In support, mean abundance did not increase from age classes 4 to 5 at Reach 2, which had the highest log mean abundance overall. However, the paucity of older fish sampled in Reach 1 and Reach 3, makes it impossible to determine if they reach a similar maximum burden.

Abundance of white grub in fish intermediate hosts can be influenced by water flow (Hunter III and Hunter 1940). It is likely that parasite burdens are influenced by seasonal environmental conditions (Noble and Noble, 1961) and seasonal differences in white grub has been shown in other studies. Steinauer and Font (2003) found a lower than expected prevalence of white grub in the spring and summer, and higher than expected prevalence in the fall and winter. Similarly, white grub prevalence and mean abundance in *Fundulus zebrinus* in the South Platter River Nebraska corresponded to changes in stream flow. Prevalence was almost 100% in the fall, when water levels were low, and once water levels rose, prevalence and mean abundance decreased (Janovy et al., 1997). Increased water flow rates may lead to parasite larvae being transported out of the system (Marcogliese, 2016). It is possible that lower water flow in the fall allowed for a larger intermediate snail host population or more efficient transmission by poorly swimming cercariae released from infected snails, or young of year fish sampled in spring were not yet infected. Alternatively, infected fish may be dying over the winter.

Effluent streams may cause drastic shifts in invertebrate communities, and decrease populations of aquatic macroinvertebrates, such as mollusks, that are sensitive to pollution (Landsberg et al., 1998). In this system, bivalve mollusks are present in Reach 1 and Reach 2, but do not live in Reach 3 (personal observation). This reach is characterized by high levels of chloride ions and very high conductivity (Kelly et al., 2012). It is likely that snail intermediate hosts may also be limited in Reach 3, accounting for lower transmission. Pulmonate snail populations generally prosper in low flow aquatic systems, allowing for trematode numbers to multiply rapidly through asexual reproduction, and then be dispersed into the water as motile larvae to infect fish (Janovy, 1987). Therefore, fish parasite abundances can be higher and more varied during the summer due to "an increase in fish feeding and the number of infective stages that the hosts come into contact with" (Dogiel, 1961). There was a drought in fall 2012, leaving Reach 2 as a series of disconnected, pooled areas. This sample exhibited the highest log mean abundance of any collection event. It is likely that a lack of flow concentrates bluegill and snail host populations and makes it easier for poorly swimming infective cercariae to find and infect a fish. From spring 2013 to spring 2014, Reaches 1 and 2 had similar patterns and showed a lower abundance in the fall when compared to spring collection events. In contrast, Reach 3 showed the opposite pattern over the same time period, and had a significantly higher log mean abundance in fall 2013 when compared to the two spring collection events. This pattern also suggests that heavily infected fish may be lost over the winter at impacted Reach 3.

White grub abundance reached over 2,000 in some bluegill. One would expect an effect on host health due to the energy reallocated to the encysted metacercariae, whether it is a decrease in body condition/gonadosomatic index, or an increase in baseline cortisol levels indicating stress. Although metacercariae were recovered from multiple organs, the liver and kidney were most commonly infected. Studies conducted by Colley and Olson (1963) and Steinauer and Font (2003) on white grub showed that the liver was the most infected organ. In this study, liver abundance in light infections (<50 total metacercariae) was over twice that of the kidney. However, in infections greater than 50 total metacercariae, abundance in the two organs was equal. It is not clear if this is due to space limitation or some other physiologic or immunologic explanation. However, the fact that the kidney can hold an equivalent number of metacercariae, in spite of it being much smaller than the liver, would argue against space limitation.

Although heavy infections of white grub in snails may be extremely pathogenic, (Bernot and Lamberti, 2008) leading to a decrease in the snails' thermal resistance, possible degradation of the liver (Noble and Noble 1982) and parasitic castration, (Sorensen and Minchella, 2001; Lockyer et al., 2004) white grub does not appear to have the same effect on the fish intermediate host. Mitchell et al. (1982) found intense infections of white grub in fathead minnows to be non-lethal and cause only a cellular inflammatory response in the organs. Likewise, in a study conducted by Lewis and Nickum (1964), metacercaria abundance did not affect the coefficient of condition as a measure of relative plumpness of bluegill longer than 4 inches. Neff and Cargnelli (2004) found Fulton's condition of bluegill was negatively correlated with parasite density, but condition was better in individuals with low levels of infection. Comparing bluegill condition indices to metacercariae log abundance at the three reaches showed there was no significant correlation with bluegill relative weight (W_r%) or gonadosomatic index (GSI) in this study. However, Fulton's condition was positively correlated with log abundance of white grub in the Sangamon River. Bluegill with less than 50 total metacercariae made up 85% of the bluegill population ≤79mm in total length assessed by Fulton's condition. In these fish, liver abundance should reliably be twice that of the kidney, and as parasite burdens increased in this lightly infected young fish, the ratio of bluegill weight to abundance also increased.

Baseline cortisol levels of bluegill did not correlate with white grub abundance in effluent impacted site reach 3 or unimpacted reach 1. In studies using baseline cortisol levels to measure stress, bluegill cortisol was unaffected by high parasitism rates, implying either metacercariae do not add measurable stress or bluegill became acclimated to this stressor and decreased their stress response (Barton et al., 1987; Pickering and Stewart, 1984; Jentoft et al., 2005).

A study on seasonal dynamics of a related species, *Posthodiplostomum cuticola,* found abundance did not decrease after the fish's first winter, suggesting infected fish had attained enough energy storage and reached a sufficient body size, regardless of parasitism, for winter survival (Ondračková et al., 2004). They also reported that heavily parasitized juvenile fish had a longer standard length and heavier body weight from July to August when compared to unparasitized individuals. They suggested *P. cuticola* may be able to manipulate its host behavior and decrease parasitized fish activity or increase motivation to forage for food, putting them closer to snail habitats.

While we were not able to discern an effect of white grub on bluegill condition within the Sangamon River, the lack of unparasitized bluegill within this system made it difficult to compare a healthy, uninfected individuals' condition to that of an infected one. However, even heavily infected fish in this river had good condition scores. We did identify parasite parameter differences among reaches. Although we did not discern a negative effect of high parasite burdens on bluegill overall, effluent impacted Reach 3 showed a significant decrease in white grub log abundance from fall to the following spring, suggesting some bluegill with higher level of infections were not able to successfully over winter. Finally, this study did not assess the first infection in young of year fish so we could not evaluate impacts of white grub alone or in conjunction with effluent impact on young of year survival. In order to more fully understand the effects of white grub infections on effluent-impacted fish, a comparison of an unimpacted river system with more uninfected bluegill would be needed.

List of Appendices

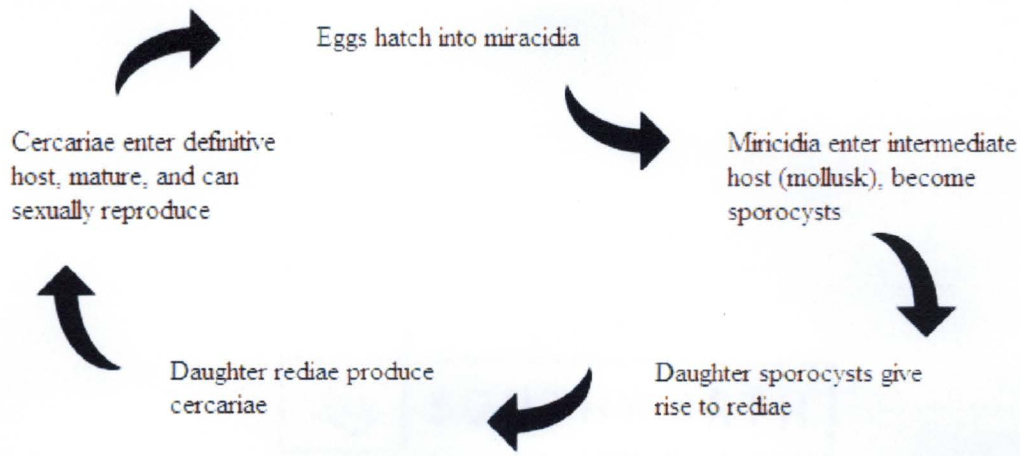


Fig.1) A typical digenetic trematode life cycle.

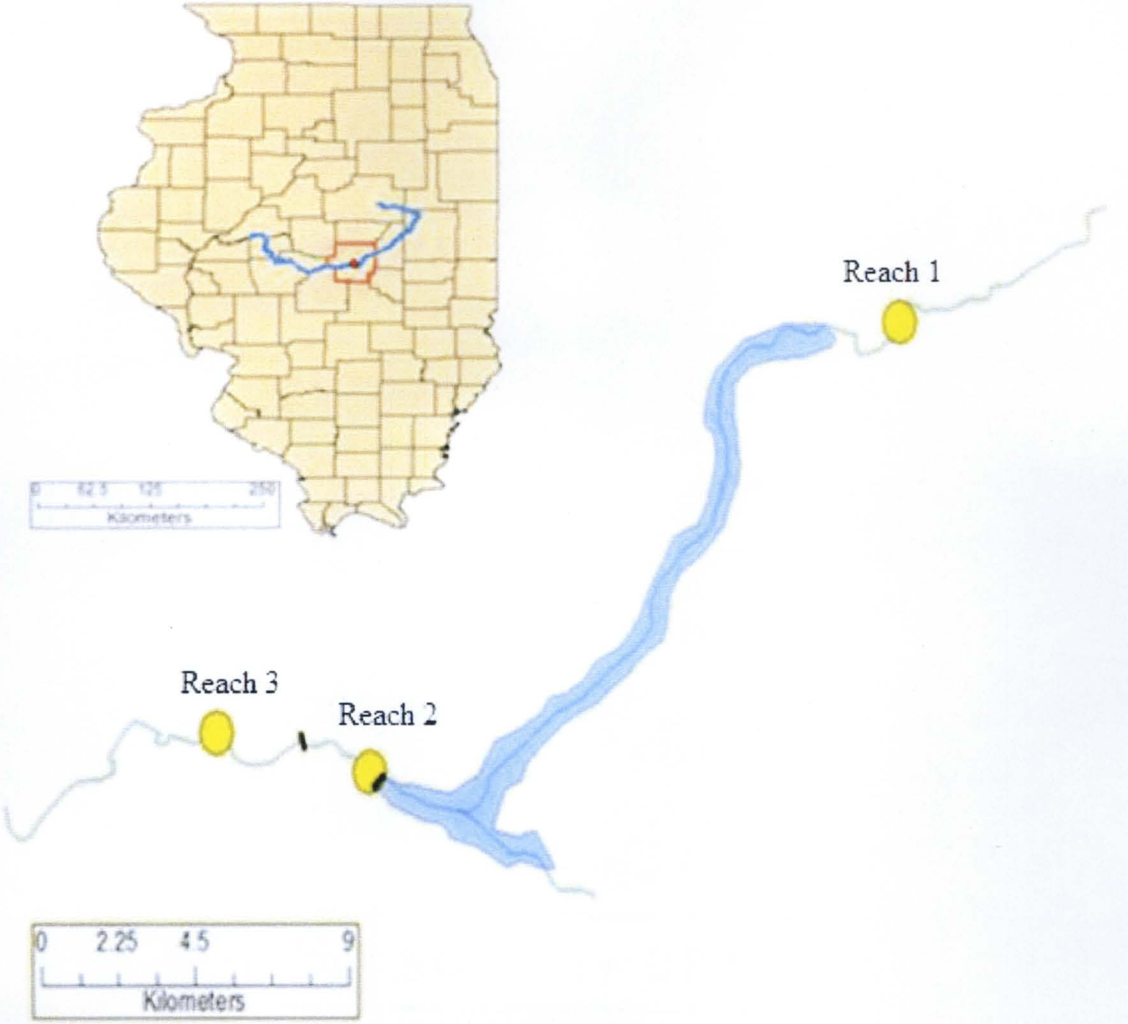


Fig. 2) Sangamon River study site locations in Decatur, Illinois.

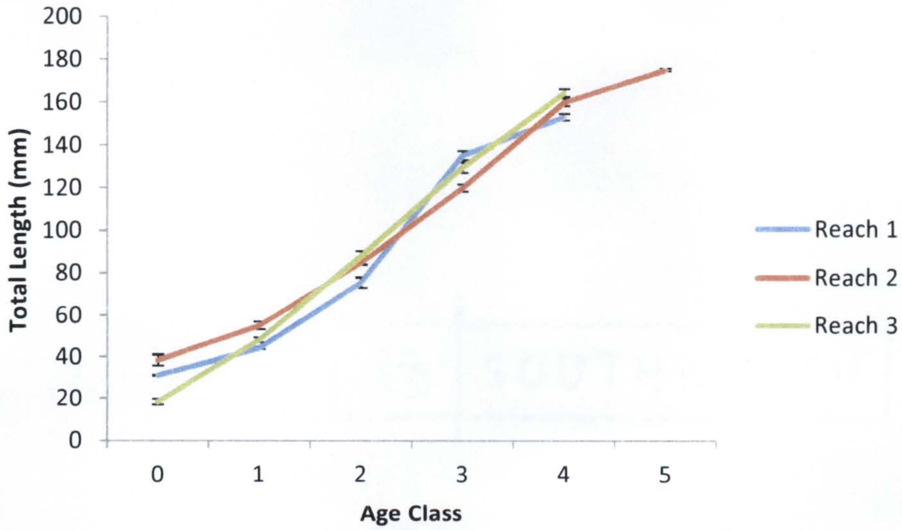
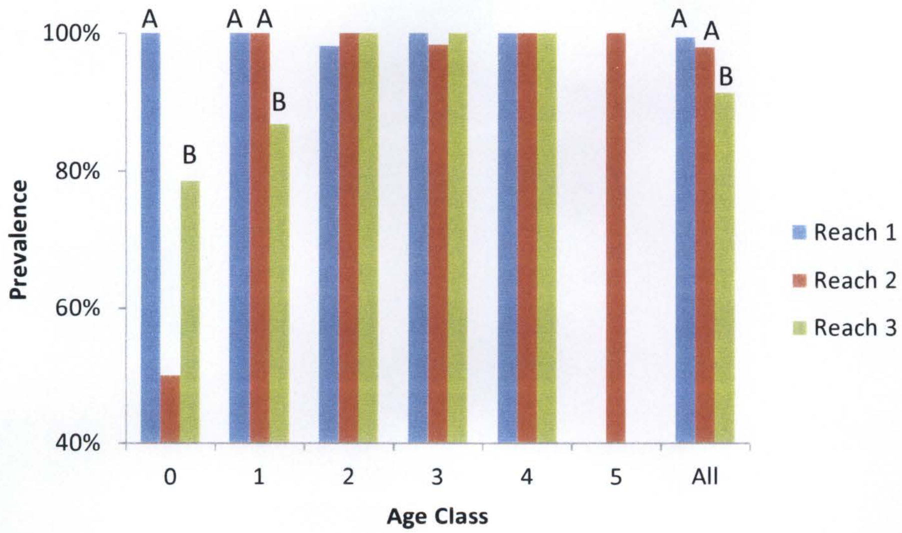


Figure 3) Average length (mm) of bluegill at each age class collected from Reach 1 (blue, N=152), Reach 2 (red, N=129), and Reach 3 (green, N=127) of the Sangamon River, Decatur, IL.



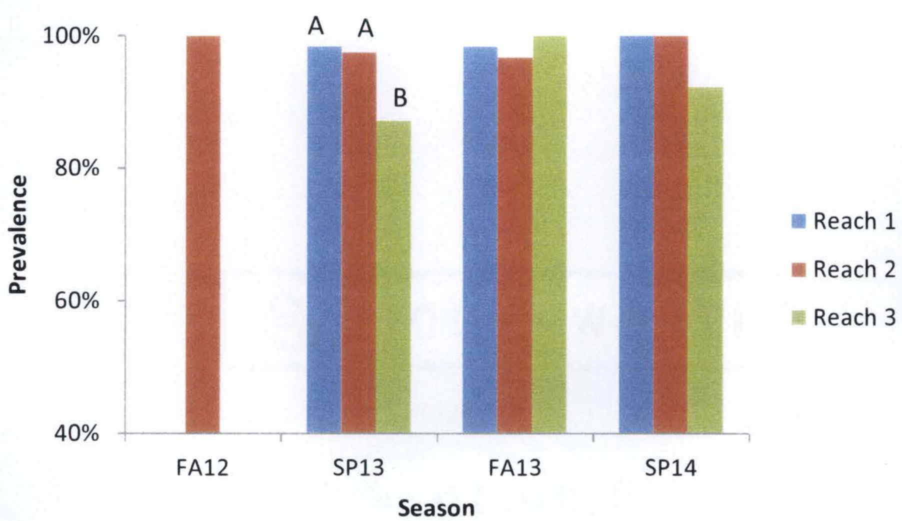


Figure 4) White grub prevalence in bluegill for age class (top) and each season (bottom) collected from Reach 1 (blue, N=152), Reach 2 (red, N=148) and Reach 3 (green, N=152) of the Sangamon River, Decatur, IL. Prevalence with same letter are not significantly different.

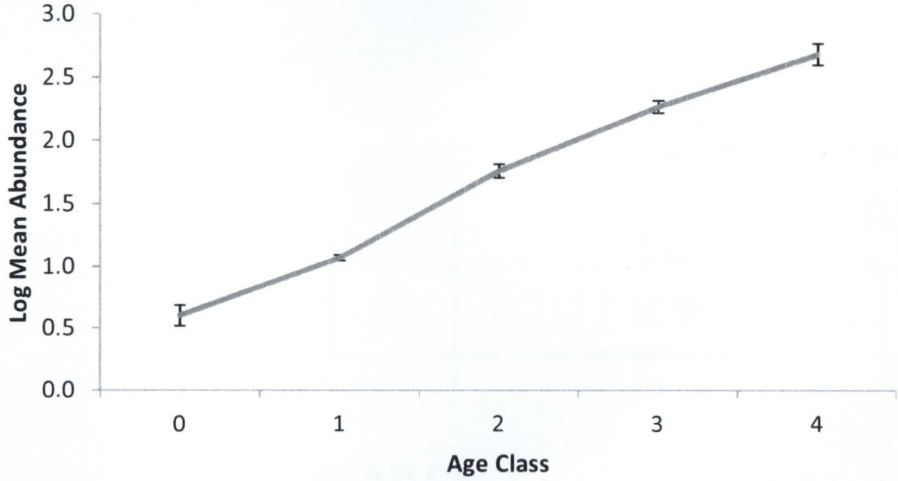


Figure 5) The relationship between bluegill age class (N=418) and white grub log mean abundance collected from Sangamon River, Decatur IL.

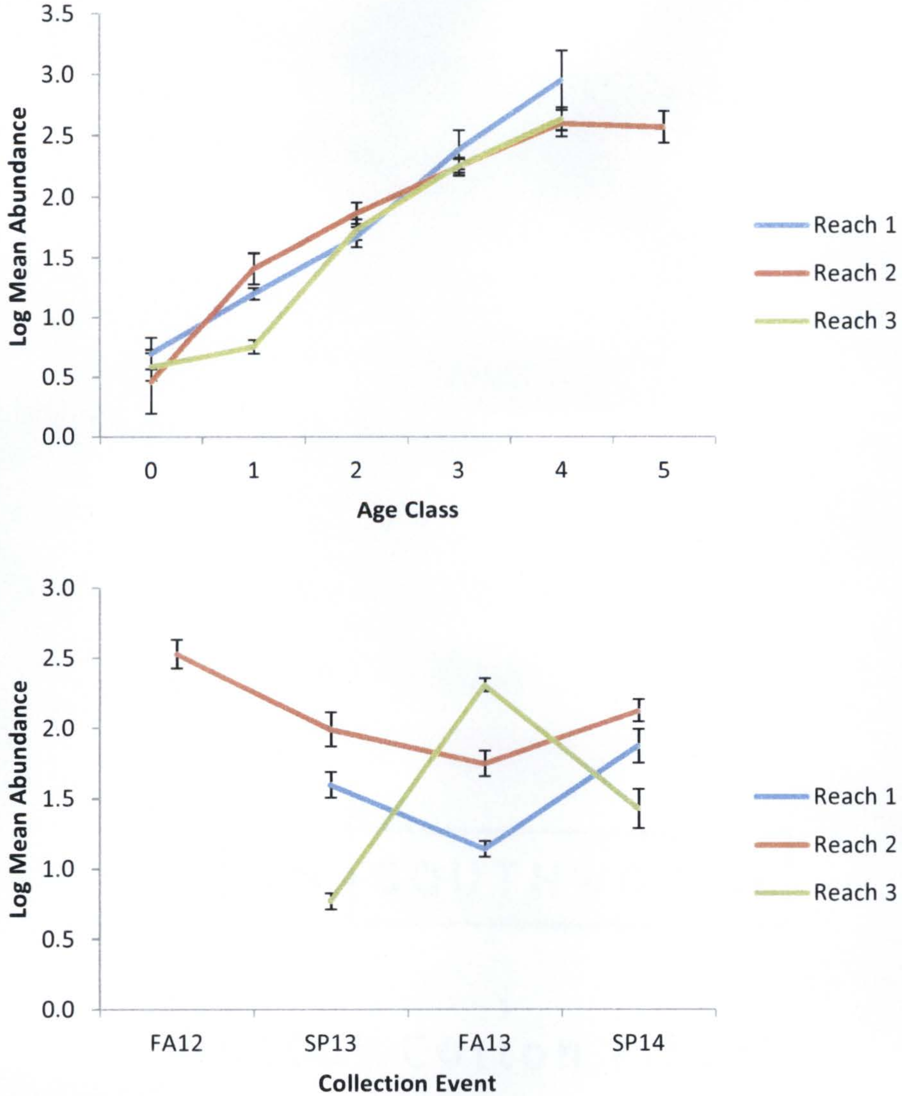
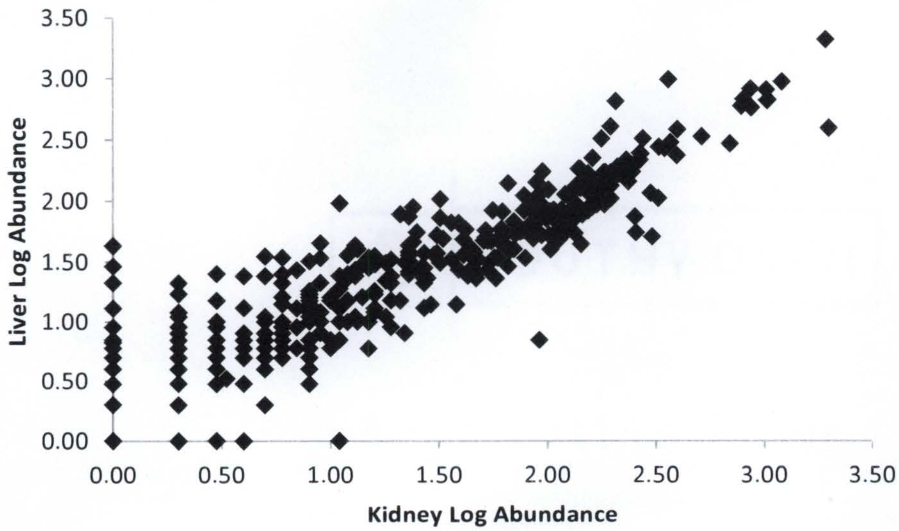


Figure 6) Log mean abundance of white grub (±SE) at each bluegill age class (top) and season (bottom) from Reach 1 (N=152), Reach 2 (N=148), and Reach 3 (N=127) of the Sangamon River, Decatur, IL



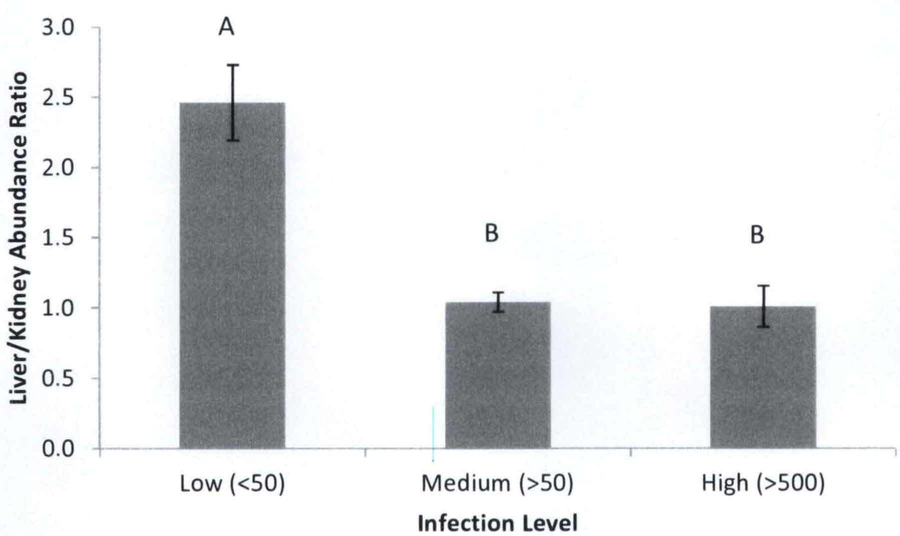


Figure 7) The correlation between liver and kidney abundance (top, N=427) and ratio of white grub abundance in low to high infection levels in all bluegill (bottom) collected from the Sangamon River, Decatur IL. Means with the same letter are not significantly different.

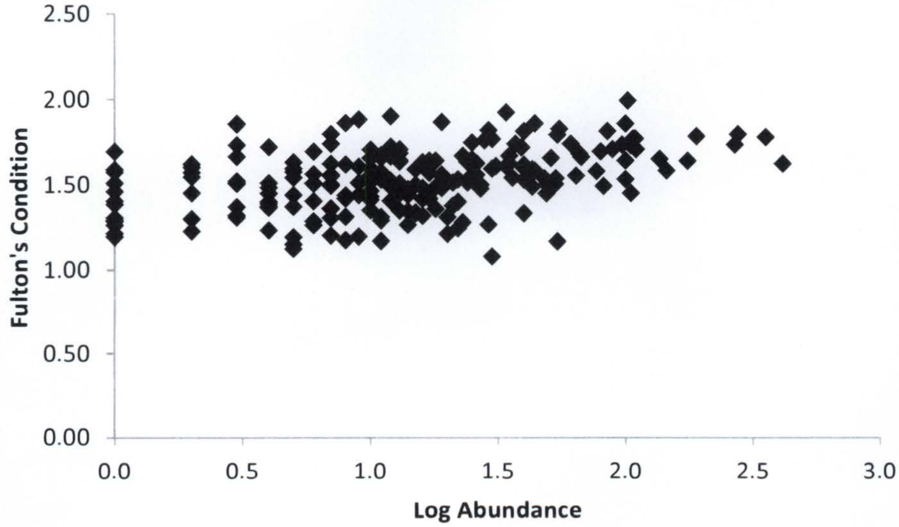
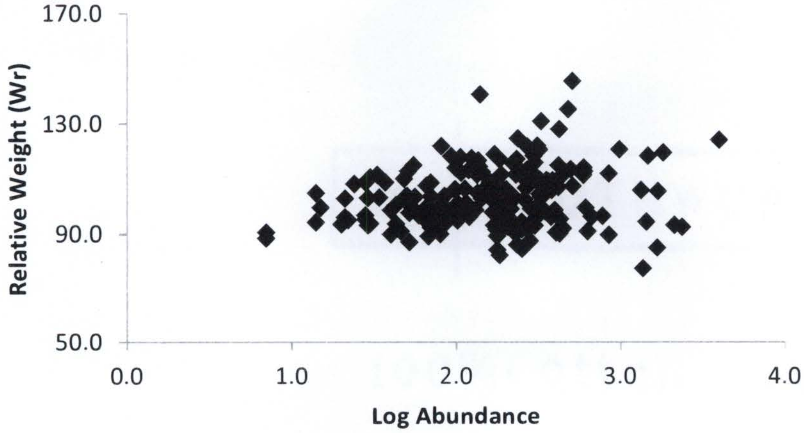
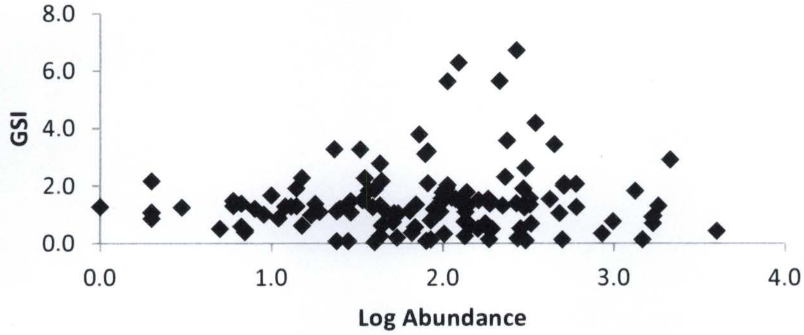


Figure 8) White grub log abundance in relation to Fulton's condition (N= 212) of bluegill collected from Sangamon River, Decatur, IL.





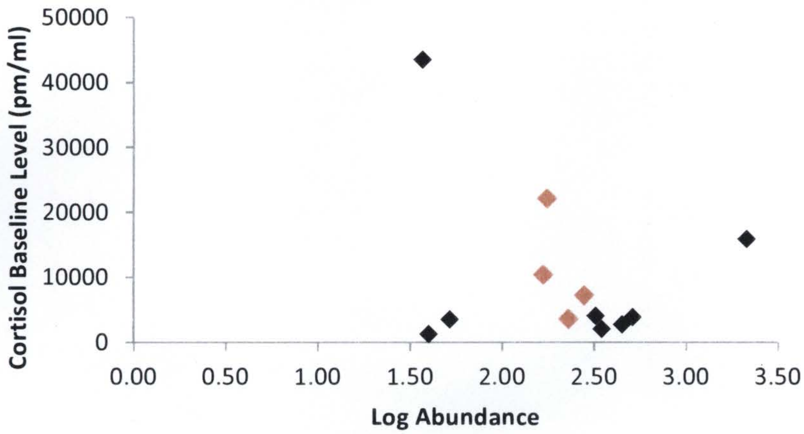


Figure 9) White grub log abundance in relation to bluegill relative weight (top, N=188), gonadosomatic index (middle, N=126) and baseline cortisol level (bottom, N= 12, data points in red represent combined serum samples) from Sangamon River, Decatur, IL.

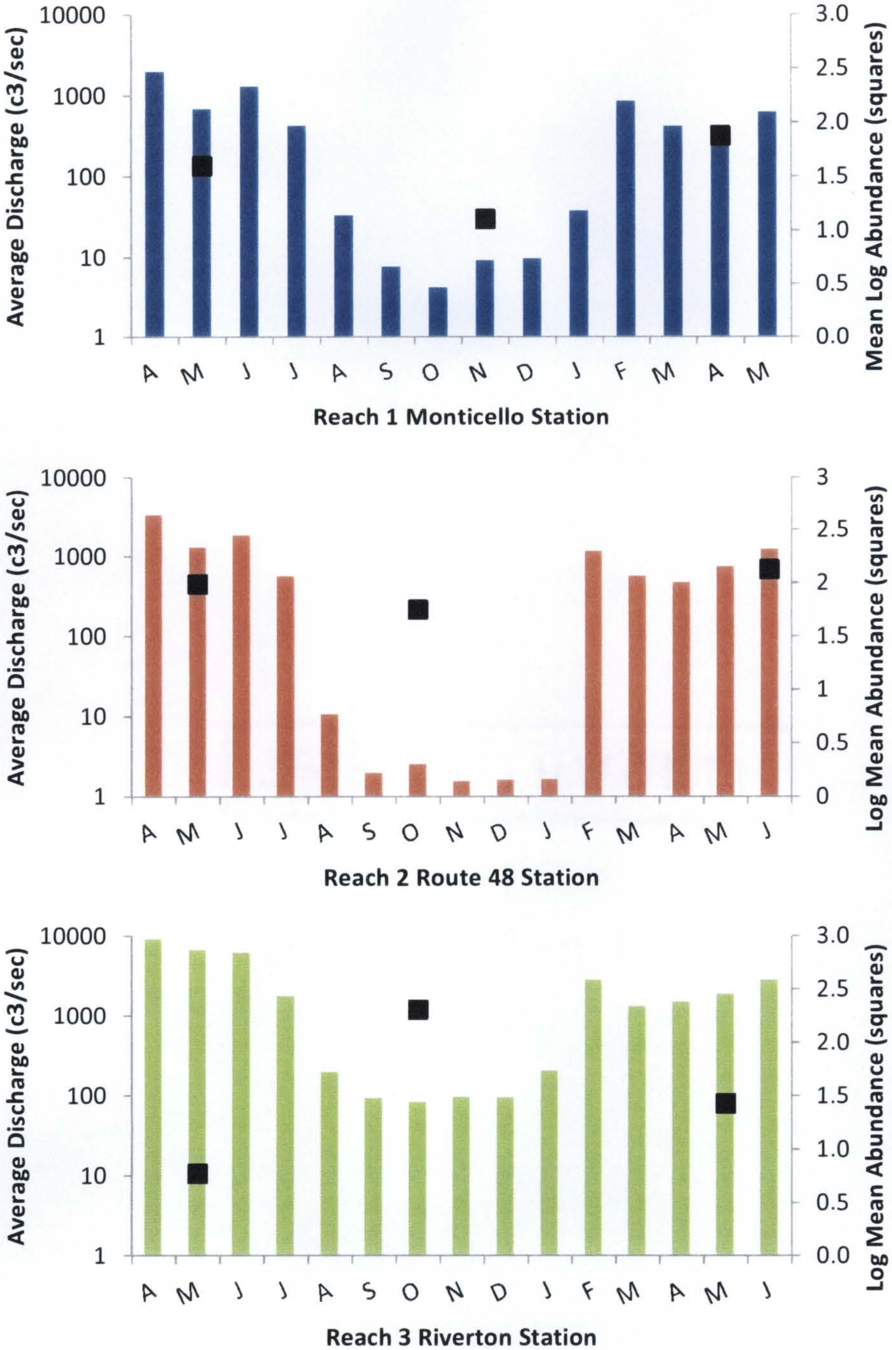


Figure 10) Average discharge (bars) in cubic ft/sec of the Sangamon River, Decatur, IL and white grub log mean abundances (squares) from 2013 to 2014.

Appendix 1. Sample size of each reach within the Sangamon River, Decatur, IL, based on bluegill age class, collection events and various bluegill condition indices.

Category	Collection Event	Reach 1	Reach 2	Reach 3
N	FA12	-	19	-
	SP13	62	39	70
	FA13	60	60	31
	SP14	30	30	26
	All	152	148	127
Bluegill Age Class	0	8	4	14
	1	76	24	61
	2	52	47	20
	3	12	58	28
	4	4	11	4
	5	0	4	0
Wr	FA12	-	10	-
	SP13	19	22	5
	FA13	0	46	31
	SP14	16	29	10
	All	35	107	46
Fulton's Condition	FA12	-	8	-
	SP13	39	12	48
	FA13	60	14	0
	SP14	14	1	16
	All	117	35	64
GSI	SP13	30	30	19
	SP14	17	21	9
	All	47	51	28

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